Inflammatogenic Properties of Bacterial DNA Following Cutaneous Exposure

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Bacterial DNA and oligodeoxynucleotides containing cytosine–phosphate–guanosine sequences and thereby mimicking prokaryotic DNA, have recently been shown to exert potent immunostimulatory properties. As skin normally harbors bacteria, and as the bacterial content and the levels of bacterial degradation products increase during skin infection, we analyzed the potential inflammatogenic role of bacterial DNA and oligodeoxynucleotides in a mouse model of cutaneous inflammation. Bacterial DNA from *Staphylococcus aureus* was injected intradermally into mice and its inflammatogenic properties were compared with synthetic phosphodiester and phosphorothioate cytosine–phosphate–guanosine- or GpC-containing oligodeoxynucleotides. A peak inflammatory infiltrate in the skin was seen already 2 d after injection with either bacterial DNA or the phosphodiester cytosine–phosphate–guanosine-oligodeoxynucleotides. In contrast, nuclease-resistant phosphorothioate cytosine–phosphate–guanosine-induced dermatitis peaked 7 d after intradermal injection. The inflammatory infiltrates consisted mainly of macrophages, and depletion of this cell population resulted in a significant ($p = 0.0001$) decrease in the severity of inflammation, which suggests that macrophages play a central part in inflammatory responses in the skin following exposure to cytosine–phosphate–guanosine-containing oligodeoxynucleotides. A significant decrease in local inflammatory infiltrate was also seen in mice with deficiencies in neutrophil or lymphocyte populations, which indicates that these cell populations may also be involved in mediating inflammatory signals after the injection of immunostimulatory DNA sequences. In summary, our results suggest that bacterial DNA is an important virulence determinant and inflammatory stimulus during skin infections. ***Key words: bacterial DNA/mice/skin/synthetic oligodeoxynucleotides. J Invest Dermatol 121:294–299, 2003***

Bacterial DNA is known to have stimulatory effects (Tokunaga *et al*, 1984; Yamamoto *et al*, 1992) on several immune cell populations, in both humans and mice. This effect has been shown to be largely dependent on cytosine–phosphate–guanosine (CpG) sequences (reviewed in Krieg, 2002) that are unmethylated at cytosine residues. This is in contrast to eukaryotic nuclear DNA, which is typically methylated at cytosine residues and has significantly fewer CpG dinucleotides compared with the bacterial genome (Bird, 1987). These differences in structure and content render eukaryotic nuclear DNA nonstimulatory. Synthetic ogliodeoxynucleotides have been used to study the *in vitro* effects of DNA on different immune cell populations, and modifications such as the incorporation of nuclease-resistant backbone structures, have been shown to amplify dramatically the immunostimulatory properties (Krieg *et al*, 1995). Inversion of the dinucleotide CpG to GpC, or alterations to the flanking sequences abrogate the immunostimulatory effect. Phosphorothioate ogliodeoxynucleotides are more resistant to degradation by nucleases compared with phosphodiester-containing ogliodeoxynucleotides, and are powerful activators of macrophages, dendritic cells, neutrophils, and B cells. Some researchers suggest that bacterial DNA should be regarded as one of the pathogen-associated molecular patterns, together with lipopolysaccharide and lipoteichoic acid (reviewed by Hacker *et al*, 2002). Immunostimulatory ogliodeoxynucleotides are optimally 20 nucleotides in length and the flanking regions are also of importance for the stimulatory effects. Specific nucleotide sequences have been shown to have optimal immunostimulatory activities for different species (Van Uden and Raz, 2000). For example, the optimal sequence for the activation of murine cells is GACGTT, and the optimal sequence for the activation of human cells is GTCGTT. Ogliodeoxynucleotides activation of immune cells can be used for its beneficial effects, e.g., as adjuvants in vaccination; however, adverse inflammatory effects that follow the administration of ogliodeoxynucleotides comprise both local and systemic reactions, including arthritis (Deng *et al*, 1999), meningitis (Deng *et al*, 2001), and life-threatening symptoms of septic shock (Sparwasser *et al*, 1997). The aim of this study was to assess the inflammatogenic potential of bacterial DNA and synthetic ogliodeoxynucleotides in the skin. The elucidation of DNA-induced pathways of inflammation is particularly relevant to dermatology since (1) bacterial infections frequently occur in the skin, and (2) synthetic oligonucleotides are frequently administered intracutaneously or subcutaneously, as part of a vaccination regimen.

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MATERIALS AND METHODS

Mice Female, 8 wk old NMRI mice and female 7 to 8 wk old BALB/C mice were purchased from B&K Universal (Sollentuna, Sweden). Female

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SCID mice and sex-matched congeneic CB17 control mice were purchased from M&B (Bomholtvej, Denmark). The mice were housed at the animal facility of the Department of Rheumatology and Inflammation Research, University of Göteborg and maintained under standard conditions of temperature and light, and fed laboratory chow and water *ad libitum*.

Oligonucleotides The nuclease-resistant, phosphorothioate–backbone ogliodeoxynucleotides and the phosphodiester-backbone ogliodeoxynucleotides that were used in this study are listed in **Table I**. All of the ogliodeoxynucleotides were synthesized by Scandinavian Gene Synthesis AB (Köping, Sweden), and shown to contain less than 5 fg of endotoxin per μg DNA, as assessed by the Limulus amoebocyte lysate assay. Genomic DNA was purified from *Staphylococcus aureus* strain LS-1 as described previously (Deng *et al*, 1999) and resuspended in phosphate-buffered saline (PBS). The DNA sample originating from *S. aureus* was shown to contain 0.04 pg of endotoxin per μg DNA (Limulus amoebocyte lysate assay, Charles River, Charleston). Nuclear DNA was extracted from nuclei isolated from NMRI mouse livers essentially as described by Wang (1967). Ten micrograms of the respective ogliodeoxynucleotide were injected intradermally on the shaved back of the mice. The oligonucleotides were diluted in PBS, and inoculated in a volume of 50 μL.

Histopathologic examination After routine procedures, tissue sections from skin samples corresponding to the injection sites were cut and stained with hematoxylin and eosin. All slides were coded and assessed in a blinded manner. The specimens were evaluated with regard to the extent of the inflammation and judged on an arbitrary scale, from grade 0 (no signs of inflammation), grade 1 (mild diffuse or focal inflammation in a single area), to grade 2 (moderate diffuse or focal inflammation), and grade 3 (heavy diffuse and focal inflammation). Examples of typical grade 0, 1, 2, and 3 reactions are presented in **Fig 1**.

Table I. List of oligodeoxynucleotide sequences used in this study

Oligodeoxynucleotides	Sequence 5′→3′
Phosphorothioate oligodeoxynucleotides	
1600	TCGTCGTTTTGTCGTTTTGTCGTT
1700	TGCTGCTTTTGTGCTTTTGTGCTT
Phosphodiester oligodeoxynucleotides	
0899	TCCATGACGTTCCTGATGCT
1800	TCCATGAGCTTCCTGATGCT
2798	TCCATGAXGTTCCTGATGCT[a]

[a]X = 5-methyldeoxycytidine.

Skin samples were also analyzed regarding the occurrence of $CD11b^+$ cells encompassing macrophages and neutrophils (macrophages expressing a single rounded nucleus and neutrophils displaying multilobular nucleus) and CD3-expressing T cells. Briefly, skin samples were frozen in isopentane prechilled with liquid nitrogen, and kept

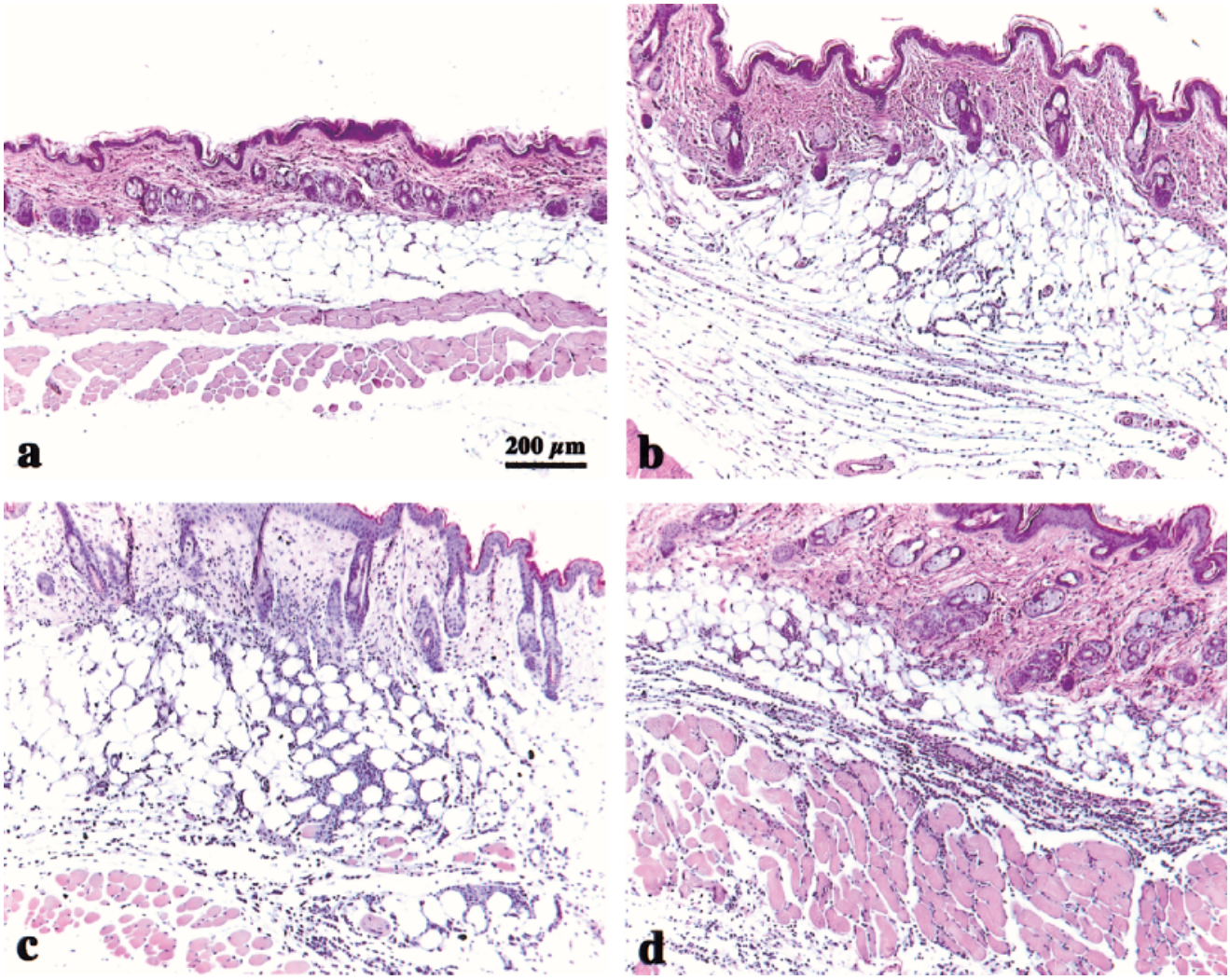

Figure 1. Grading of severity of cutaneous inflammation following injection with DNA. Representative photomicrographs of skin biopsies (stained with hematoxylin/eosin) represent different degrees of inflammatory infiltrates. (*a*) no inflammation (grade 0); (*b*) mild diffuse or focal inflammation in a single area (grade 1); (*c*) moderate diffuse or focal inflammation (grade 2); and (*d*) heavy diffuse and focal inflammation in an extended pattern (grade 3). In (*a*) 10 μg of phosphorothioate-GpC was injected, in (*b–d*) 10 μg of phosphorothioate-CpG was injected. All samples were collected at day 7 after injection.

at −70°C until cryosectioned. All of the sections were fixed in cold acetone for 5 min and washed in PBS. The sections were incubated overnight in a humid atmosphere at +4°C with unlabeled rat anti-CD11b (Mac-1; M1/70) (Springer *et al*, 1979) or rat anti-CD3 (clone 17A2 PharMingen, San Diego, California) monoclonal antibodies (MoAb), which were diluted in PBS containing 1% bovine serum albumin. After several washes, endogenous peroxidase was depleted by treatment with 0.3% H_2O_2 for 5 min biotin-labeled rabbit anti-rat immunoglobulin (Vector Laboratories, Burlingame, California) diluted in PBS/bovine serum albumin were used as secondary anti-bodies. The binding of biotin-labeled secondary antibodies was detected by stepwise incubation with streptavidin–biotin complex/horseradish peroxidase (DAKO, Glostrup, Denmark) and 3-amino-9-ethyl-carbazole containing H_2O_2. All sections were counterstained with Meyer's hematoxylin.

Monocyte/macrophage depletion Etoposide (Bristol-Myers Squibb AB, Bromma, Sweden) is a cytotoxic drug that is known to deplete selectively the monocyte/macrophage populations in rabbits and mice (Van't Wout *et al*, 1989). Etoposide acts by inhibiting the function of DNA topoisomerase II, and thus interrupts the late S/G_2 phase of the cell cycle (Smith *et al*, 1994). Etoposide was diluted 1:10 in PBS (0.13 M NaCl, 10 mM sodium phosphate (pH 7.4)) from a stock solution of 20 mg per mL. A volume of 120 μL, which corresponded to 12.5 mg per kg body weight of etoposide, was injected subcutaneously (s.c.) in BALB/C mice on 2 consecutive days before, and on 5 consecutive days after, intradermal injection of the phosphorothioate ogliodeoxynucleotide. The dose of etoposide was chosen according to that established in earlier studies (Calame *et al*, 1994). Fluorescence-activated cell sorter analysis of peripheral blood was performed to assess the effect of macrophage depletion, as previously described in detail (Verdrengh and Tarkowski, 2000), and showed that etoposide depleted the monocyte population by 80%. Control BALB/C mice received the same volume of PBS intraperitoneally (n = 5) or subcutaneously (n = 5).

Neutrophil depletion MoAb RB6-8C5 is a rat immunoglobulin G2b (IgG2b) antibody that selectively binds to and depletes mature mouse neutrophils and eosinophils in BALB/C but not NMRI strain of mice. Hybridoma cells secreting RB6-8C5 were a kind gift from R. Coffman (DNAX Research Institute, Palo Alto, California). The hybridoma cells were expanded in Iscove's medium (Gibco, Paisley, UK) supplemented with 5% heat-inactivated fetal bovine serum (Seralab, Crawley Down, UK), 50 μg gentamicin per mL, 2 mM L-glutamine, and 5×10^{-5} M β-mercaptoethanol. The cells were grown to maximum density and the immunoglobulins were precipitated with 50% saturated ammonium sulfate, dialyzed against PBS, and filter sterilized. The concentrations of immunoglobulins were determined by the radial immunodiffusion method (Mancini *et al*, 1965). As a control, monoclonal immunoglobulin-class-matched anti-ovalbumin antibodies (anti-OVA MoAb) were used (kindly provided by Dr Telemo, Department of Rheumatology and Inflammation Research, University of Göteborg, Sweden).

Analysis of peripheral blood by fluorescence-activated cell sorter has previously shown depletion of the granulocyte population by more than 90% within 48 h (Deng *et al*, 2000; Molne *et al*, 2000). BALB/C mice were injected intraperitoneally with one mg of either the MoAb RB6-8C5 or the class-matched anti-OVA MoAb 2 h before, and 2 and 5 d after intracutaneous injection of the CpG-ogliodeoxynucleotide.

Interleukin (IL)-6 analysis Serum IL-6 levels was assessed to measure a possible systemic effect of the oligonucleotide administered. The cell line B13.29, which is dependent on IL-6 for growth, has been described previously (Lansdorp *et al*, 1986; Aarden *et al*, 1987; Helle *et al*, 1988). For IL-6 determinations, the more sensitive subclone B9 was used. B9 cells were harvested from tissue culture flasks, seeded on to microtiter plates (Nunc, Roskilde, Denmark) at a concentration of 5000 cells per well, and cultured in Iscove's medium supplemented with 5×10^{-5} M 2-mercaptoethanol, 10% fetal bovine serum gentamycin (50 μg per mL), and L-glutamine. The serum samples were added for 68 h and [^{3}H]thymidine was added 4 h prior to harvesting. Each sample was tested for IL-6 in a series of 2-fold dilutions and compared with a recombinant IL-6 standard. B9 cells do not react with recombinant cytokines, such as IL-1α, IL-1β, IL-2, IL-3, IL-5, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-α, and interferon-γ and have only weak reactivity with IL-4 (Helle *et al*, 1988).

Experimental protocol NMRI mice were injected intradermally with either phosphorothioate CpG-ogliodeoxynucleotide or GpC-ogliodeoxynucleotide and serum samples were taken for analysis of IL-6 levels at days 0, 2, 7, and 21. Skin samples for histopathologic analysis of the size and density of the inflammatory infiltrate were obtained at days 2, 7, and 21. NMRI mice were also injected with phosphodiester CpG-ogliodeoxynucleotide or GpC-ogliodeoxynucleotide as well as DNA from *S. aureus* LS-1 strain. Skin samples were obtained for histopathologic analysis on days 2 and 7 postinoculation. SCID mice and their controls (CB17) were injected intradermally with phosphorothioate CpG-ogliodeoxynucleotide and skin samples for histopathologic analysis were obtained at day 7. The BALB/C mice were injected with phosphorothioate CpG-ogliodeoxynucleotide and with etoposide, RB6-8C5, control MoAb, or PBS, respectively. Skin samples for histopathologic analysis were obtained 7 d after injection of the ogliodeoxynucleotide.

Statistical analysis The differences between mean values were tested for statistical significance using the nonparametric Mann–Whitney U test. $p \leq 0.05$ was considered to be statistically significant.

Ethics This study was approved by the local ethics committee for animal use (registration number 175–2000) at Göteborg University.

RESULTS

Nuclease-resistant, phosphorothioate-modified CpG oligonucleotide (oligonucleotide 1600) induced inflammation in the skin of NMRI mice at doses of 50 μg as well as 10 μg. As no major differences were observed in either the size or density of the inflammatory infiltrate, a dose of 10 μg of oligonucleotide was used consistently throughout this study. An inflammatory infiltrate equal to grade 2 or 3 frequently included all layers of the skin and often engaged not only subcutaneous fat and dermis, but also the superficial muscle. The inflammatory infiltrate consisted mainly of macrophages, whereas neutrophils were less frequent. Lymphocytes occurred sparsely in all the skin biopsies. These findings were confirmed with immunohistochemistry.

Inflammatogenic properties of phosphodiester–ogliodeoxynucleotide and phosphorothioate-ogliodeoxynucleotide in murine skin Nuclease-sensitive, phosphodiester CpG-oligonucleotide (oligonucleotide 0899) triggered inflammatory changes in NMRI mice with peak values 2 d after injection and almost complete resolution 7 d after injection (**Fig 2**). The

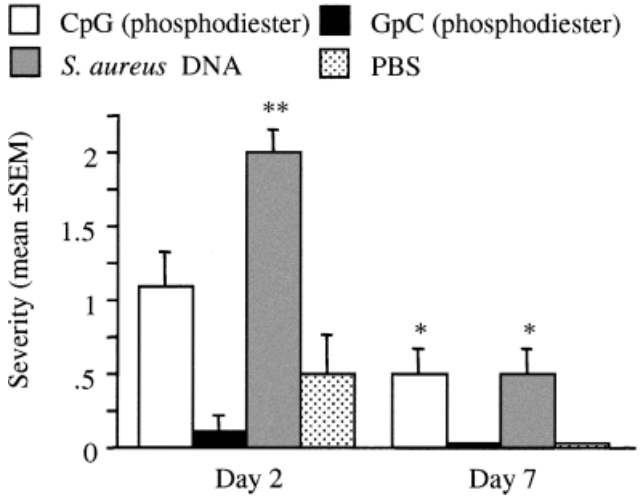

Figure 2. Inflammatory responses triggered by bacterial DNA and phosphodiester oligonucleotides. Nuclease-sensitive bacterial DNA from *S. aureus* LS-1 triggers heavy inflammatory changes in skin within 2 d following injection, whereas the GpC-ogliodeoxynucleotide is inert. On day 7 the inflammatory responses have resolved partially, with absence of inflammation in mice injected with PBS or GpC-ogliodeoxynucleotide, but with still noticeable inflammation in mice injected with bacterial DNA and CpG-ogliodeoxynucleotide (n = 9–10 in all groups) (**$p < 0.01$; *$p < 0.05$).

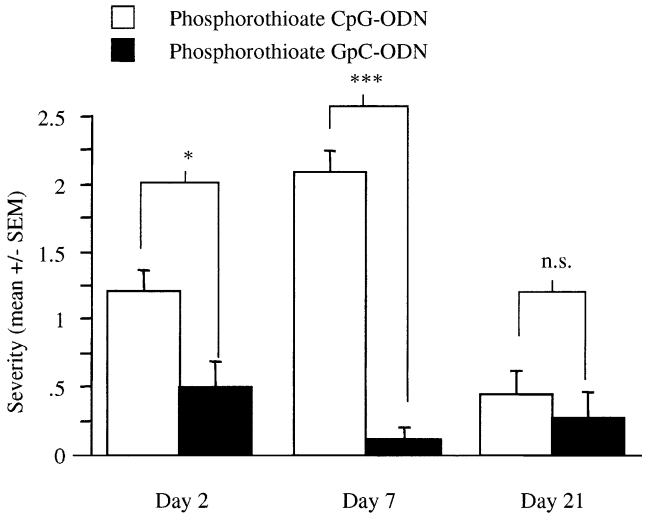

Figure 3. Phosphorothioate CpG-ogliodeoxynucleotide but not GpC-oligonucleotide triggers cutaneous inflammation. Significant local skin inflammation is evident following intradermal injection of 10 μg of phosphorothioate CpG-ogliodeoxynucleotide compared with 10 μg of phosphorothioate GpC-ogliodeoxynucleotide. Pooled data from three different experiments are shown (n = 8–10, day 2; n = 18–19, day 7; and n = 7–9, day 21) (*$p < 0.05$; ***$p < 0.001$; n.s., not statistically significant).

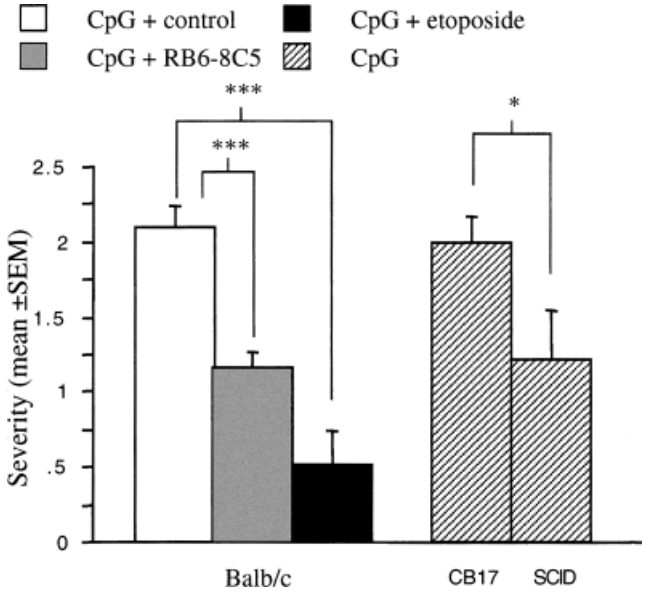

Figure 4. Depletion of immune cells results in a significant decrease in the severity of skin inflammation induced by phosphorothioate CpG-ogliodeoxynucleotide. BALB/C mice and CB-17 SCID mice as well as their controls (CB17) were injected intradermally with 10 μg of phosphorothioate CpG-ogliodeoxynucleotide. Depletion of monocytes/macrophages (n = 10) by etoposide as well as depletion of neutrophils (n = 19) by anti-neutrophilic antibody RB6-8C5 resulted in a significant decrease of the severity of the inflammation (***$p < 0.0001$), compared with BALB/C mice injected with CpG and relevant control (OVA MoAb; n = 10 or PBS; n = 11). Results from both control groups are pooled, as no differences were seen between the groups. Also, the ogliodeoxynucleotide-induced inflammatory infiltrates in SCID mice (n = 9) and their controls (CB17) (n = 9) were significantly different after injection with the same dose (10 μg) of CpG-ogliodeoxynucleotide (*$p < 0.05$). All samples are collected at day 7 following injection with CpG-ogliodeoxynucleotide.

injection of bacterial DNA (*S. aureus* LS-1) in NMRI mice displayed the same pattern of inflammation, with peak values 2 d after injection (**Fig 2**). Furthermore, the inflammation was more pronounced in the bacterial-DNA injection group. In contrast, injection of phosphodiester GpC-oligonucleotide (oligonucleotide, 1800) did not trigger inflammation (**Fig 2**). Methylated phosphodiester CpG-ogliodeoxynucleotide (ogliodeoxynucleotide 2798) did not induce any inflammation in seven of nine NMRI mice, and slight (grade 1) inflammation in two of nine mice 7 d after injection. Nuclease-resistant, phosphorothioate-modified CpG-oligonucleotide induced inflammatory changes in NMRI mice that peaked at day 7, with almost total resolution at day 21 (**Fig 3**). The few remaining inflammatory cells present on day 21 were mostly of monocyte/macrophage origin.

For the control purpose, we have also assessed the impact of homologous DNA (mouse liver DNA) on the induction of skin inflammation. Our results show clearly that NMRI mice injected intracutaneously with 10 μg of highly purified nuclear mouse liver DNA will not rise any inflammatory response (day 2: one of 10 mice displayed grade 1 inflammation; day 7: none of 10 mice displayed inflammation).

Effects of depletion of immune cell populations on oligonucleotide-induced skin inflammation The presence of macrophages has been shown to be mandatory for the development of arthritis following intra-articular injection of CpG-oligonucleotide, whereas neutrophils, natural killer cells, T cells, and B cells played minor parts in this type of inflammation (Deng *et al*, 2000). We investigated whether this pattern was also valid for DNA-triggered inflammation of the skin. BALB/C mice were injected intradermally with 10 μg of CpG-ogliodeoxynucleotide (ogliodeoxynucleotide 1600) and administered with (1) the macrophage-depleting drug etoposide (n = 10), or (2) the anti-granulocytic antibody RB6-8C5 (n = 19), or (3) PBS or immunoglobulin class-matched IgG antibodies specific for ovalbumin (n = 21). SCID mice (n = 9), which lack both T and B cells and their congeneic (CB17) controls (n = 9) were also injected with 10 μg of CpG-ogliodeoxynucleotide (ogliodeoxynucleotide 1600). All of the mice were killed 7 d after injection and skin samples were obtained for histopathologic examination and grading (see *Materials and Methods* section). Depletion of monocytes/macrophages with etoposide resulted in a significant reduction in the severity of inflammation in the skin ($p = 0.0001$). A statistically significant ($p = 0.0001$) decrease in the inflammatory infiltrates was seen also in mice that were treated with anti-granulocytic antibodies (**Fig 4**). Also, significant differences of low degree ($p < 0.05$) were seen in the severity of the inflammatory infiltrate in SCID mice compared with controls (**Fig 4**).

Serum IL-6 levels Serum levels of IL-6 have been shown to increase transiently after intra-articular injection of CpG-ogliodeoxynucleotide (Deng and Tarkowski, 2000). Intracutaneous injection of DNA led to a slight, but not statistically significant, increase in serum levels of IL-6 2 d after intradermal injection of 10 μg of phosphorothioate CpG-ogliodeoxynucleotide (58 ± 10 pg per mL on day 0 *vs* 163 ± 52 pg per mL on day 2 (mean ± SEM); $p = 0.06$). There were no statistically significant differences in serum IL-6 levels in these groups compared with groups of mice injected with the same dose of GpC-ogliodeoxynucleotide (data not shown).

DISCUSSION

The skin is frequently exposed to both bacterial DNA, e.g., during cutaneous infections such as erysipelas and impetigo, and to synthetic phosphorothioate oligonucleotide, as in adjuvants and various vaccination regimens. We have previously shown that

bacterial DNA, in contrast to eukaryotic nuclear DNA, exerts inflammatory effects in joints (Deng *et al*, 1999) and in the central nervous system (Deng *et al*, 2001). Similar findings have also been reported by others (Schluesener *et al*, 2001; Takeshita *et al*, 2001; Zeuner *et al*, 2002). The immunostimulatory effects of bacterial-like CpG oligonucleotides require DNA uptake and endosomal acidification (reviewed in Krieg, 1999), and are mediated through Toll-like receptor 9, which recognizes a specific pattern in the bacterial DNA (Hemmi *et al*, 2000). As the skin is commonly the site of bacterial infections and almost exclusively the site of vaccination procedures, we felt that the question related to inflammatogenicity of prokaryotic DNA sequences in this compartment should be addressed. Our results indicate both similarities and differences between bacterial DNA inflammatogenicity in the skin as compared with the joints.

In accordance with previous results, (Deng *et al*, 1999) monocytes/macrophages played a central part in mediating skin inflammation following intracutaneous injection of bacterial DNA. Indeed, both histologic stainings and immunohistochemistry of skin samples showed a plethora of macrophages that had infiltrated all layers of the skin. In addition, pretreatment of mice with a topoisomerase II inhibitor (etoposide), which leads to selective apoptosis of monocytic cells, resulted in significant downregulation of the inflammatory response of skin to DNA. Thus, this cell population and its soluble products (e.g., cytokines, chemokines, nitric oxide) are crucial to the observed inflammatory response to DNA. This finding is corroborated by the recent study of von Stebut *et al* (2002), where stimulation of skin-derived macrophages with CpG-ogliodeoxynucleotide resulted in induction of IL-12, interferon-γ, and nitric oxide resulting in killing of *Leishmania* parasites. Interestingly, and in contrast to previous findings in DNA-injected joints, neutrophils also participated in DNA-triggered skin inflammation. Indeed, depletion of this cell population with a specific MoAb led to a significant downregulation of the inflammatory responses in skin. It should be pointed out that in order to assess the significance of neutrophils with regard to bacterial DNA-triggered skin inflammation we needed to use another strain of mice (BALB/C), known to be sensitive to the depletion procedure. Recent work from our laboratory (Bylund *et al*, 2002) showed that phosphorothioate CpG-oligonucleotide activation of neutrophils led to respiratory burst and degranulation of both specific and gelatinase granules.

In contrast to the active roles of monocytes/macrophages and neutrophils in bacterial DNA-triggered skin inflammation, it is clear that the acquired immune system does not as vividly participate in this process. SCID mice, which lack functional T/B lymphocytes, showed somewhat less severe inflammation compared with those of the congeneic CB17 strain. Most probably, this finding is due to the lack of B cells, known to be activated by bacterial DNA (Yi *et al*, 1998). In this study, we showed that both phosphodiester-backbone DNA and phosphorothioate-backbone DNA were able to trigger skin inflammation; however, the phosphodiester-oligonucleotide, as well as staphylococcal DNA-triggered inflammatory responses were less durable compared with those induced by synthetically modified phosphorothioate-backbone oligonucleotides.

What is the biological significance of our findings? It has been suggested that local intradermal injection of CpG ogliodeoxynucleotide triggers an early host response to a simulated bacterial invasion, as indicated by increased migration of Langerhans cells from the epidermis (Ban *et al*, 2000). Different routes of administration of CpG-ogliodeoxynucleotide may lead to differential activation patterns of immune cells, e.g., predominantly polymorphonuclear granulocytes in the case of inflammation in the lower respiratory tract (Schwartz *et al*, 1997). Systemic, rather than local administration of CpG-ogliodeoxynucleotide, led to down-modulation of inflammation in an experimental model of asthma (Kline *et al*, 1998). The potential of immunostimulatory DNA sequences to trigger a T helper 1-polarized response is well documented and might be beneficial in chronic skin diseases that have T helper 2-type immune reactivity, such as pemphigus, pemphigoid, and cutaneous lupus erythematosus. Altogether our results indicate that both bacterial DNA and synthetic ogliodeoxynucleotide trigger local skin inflammation. Limited, local inflammation of this type might be advantageous for the host in allowing it to mobilize an efficient protective response. Under certain circumstances, however, this might induce or aggravate chronic skin inflammatory diseases, such as eczema and psoriasis.

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